# High Molecular Weight Factor V of Bovine and Human Plasma†

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ABSTRACT: Inclusion of high levels of diisopropyl fluorophosphate (DFP) in oxalated bovine blood during the time of collection at the slaughterhouse resulted in the isolation of a high molecular weight factor V. At concentrations of 2 mM DFP, the apparent size of this molecule ranged from a molecular weight of 800 000 to 1 000 000 as determined by column chromatography with Bio-Gel A-5m and by gradient pore polyacrylamide slab gel electrophoresis under "native" conditions. Failure to include 2 mM DFP at the initial blood collection step led to the recovery of lower molecular weight factor V, i.e., less than 500 000, even in the presence of benzamidine, phenylmethanesulfonyl fluoride, or soybean trypsin inhibitor. In the case of human blood, the manner of collection, i.e., venipunture, allowed repeated recovery of factor V of molecular weight in excess of 800 000. Gradient pore polyacrylamide gel electrophoresis confirmed the high molecular weight character of this factor V. A similar result was obtained for the factor V isolated from bovine blood collected by venipuncture. Establishment of the high molecular weight character of the factor V in bovine as well as human venipuncture (oxalated) blood, where no protease inhibitors were added, was greatly aided by the availability of Bio-Gel A-5m gel media. Both the human as well as the bovine high molecular weight factor V could be activated by incubation with thrombin or a purified activator protein from Russell's viper venom. These data are discussed in reference to previous reports on the occurrence of lower molecular weight forms of factor V, i.e., 500 000 or less, and it was concluded that a tissue protease was the responsible agent for formation of these latter species.

The precise role of factor V in the blood coagulation process in mammals has not been defined primarily due to the difficulties associated with its isolation and purification, and as a consequence, any detailed physicochemical characterization has not been easily achieved (Baugh & Hougie, 1977). In part, this can be attributed to the apparent inherent instability of this coagulant protein which has placed constraints on any serious study of its biochemical behavior. This situation is particularly well illustrated by an examination of the reported values for apparent molecular weight of factor V (bovine) which have ranged from 97 000 to 430 000 (Aoki et al., 1963; Smith & Hanahan, 1976). In an interesting report, Colman and his associates (Saraswathi et al., 1978) noted a significant difference in the molecular weight of factor V isolated from bovine blood collected by venipuncture, i.e., a value of $1.2 \times 10^6$ as determined on Sepharose 4B, and that of factor V isolated from blood collected at the slaughterhouse, i.e., 560 000. They also reported that factor V isolated from venipuncture blood was activated 18-fold by thrombin whereas that isolated from a slaughterhouse sample was activated only some 6-fold by thrombin. Though these investigators did not add inhibitors at the time of collection, they proposed that proteolysis could explain the occurrence of the lower molecular weight form, i.e., 560 000. Recently, publications have appeared in which new procedures were outlined for the isolation and characterization of native factor V from bovine blood. As an example, Nesheim et al. (1979) reported that through the judicious use of inhibitors (0.02% STI,[1] 10 mM benzamidine hydrochloride, and 0.1 mM DFP) they were able to isolate a single-chain polypeptide with factor V activity of a molecular weight of 330 000. In another report, Esmon (1979) described the isolation of a high molecular weight factor V of 850 000. However, most of his discussion on native factor V centered on the fact that it yielded two components of molecular weights 210 000 and 115 000 on sodium dodecyl sulfate electrophoresis when treated with thrombin.

In an ongoing study designed to investigate the biochemical nature of the factor V, we noted that this coagulant protein isolated from human blood could be activated some 50–60-fold by thrombin whereas factor V isolated from bovine (slaughterhouse) blood by a comparable procedure (Smith & Hanahan, 1976) could be activated only 15–20-fold. This difference in activation of these two sources of factor V prompted us to investigate the possibility that the bovine factor V, which has an apparent molecular weight near 430 000, as isolated by the procedure of Smith & Hanahan (1976), was actually a degradation product of a much larger molecular weight form. Inasmuch as this laboratory had shown that factor V was insensitive to the potent serine protease inhibitor DFP (Bartlett et al., 1978), it was feasible to examine the influence, if any, of proteases on the resulting molecular weight of factor V isolated from bovine blood through inclusion of serine protease inhibitors, such as DFP, at specific stages of the isolation procedure. The results of this study are reported here.

## Materials and Methods

*Reagents.* Diisopropyl fluorophosphate, soybean trypsin inhibitor (Type II-s), *p*-(chloromercuri)benzoic acid, phenylmethanesulfonyl fluoride (PMSF), and heparin (porcine, sodium salt) were purchased from Sigma Chemical Co. Benzamidine hydrochloride was acquired from Aldrich Chemical Co. Bio-Gel A-5m (100–200 mesh) was obtained from Bio-Rad Laboratories. The QAE-cellulose was a product from Schleicher and Schuell, Inc. (Lot 3118). The standards used for the column calibration and their molecular weights were as follows: Blue Dextran (Pharmacia), $2 \times 10^6$; bovine thyroglobulin (Sigma), 680 000; ferritin (Boehringer-Mannheim, Pharmacia), 440 000; catalase (Pharmacia), 232 000; aldolase (Pharmacia), 159 000. Standards for electrophoresis were purchased from Pharmacia Fine Chemicals. Their molecular weights were as follows: thyroglobulin, 667 000; ferritin, 440 000; catalase, 232 000; lactate dehydrogenase,

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[1] Abbreviations used: DFP, diisopropyl fluorophosphate; STI, soybean trypsin inhibitor; PMSF, phenylmethanesulfonyl fluoride; QAE-cellulose, 2-(hydroxypropyl)aminoethylcellulose; PEG, poly(ethylene glycol); TCF, thin channel ultrafiltration flow cell; RVV-V, factor V activator of Russell's viper venom.

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140 000; bovine serum albumin, 67 000. Bovine $\alpha$-thrombin was the generous gift of Dr. Walter Kisiel. Poly(ethylene glycol) 4000 from Baker Chemical Co. was dissolved with hot acetone and recrystallized at 0 °C by addition of one-third volume of petroleum ether. Russell's viper venom was purchased from Miami Serpentarium.

Factor V deficient plasma was prepared by incubation of human plasma, derived from blood collected in 0.1 volume of 0.1 M sodium oxalate, for 30 h at 37 °C. A factor V clotting time of greater than 60 s for the incubated, undiluted plasma reflected a factor V deficiency. A factor V standard was prepared by pooling oxalated plasma from six human donors for use on the same day. One unit of factor V activity is considered to be equivalent to that amount of factor V contained in 1 mL of pooled plasma. Oxalated blood is used for a factor V standard since blood collected in citrate (ACD) showed a very rapid decrease in factor V activity and this was not our experience with oxalate as the anticoagulant.

Thromboplastin was prepared from baboon brain acetone powder according to the procedure of Biggs (1972) with the following modifications. Three grams of baboon brain acetone powder was suspended in 100 mL of 0.5 M NaCl and warmed at 37 °C for 20 min with stirring. Two milliliters of the stirred suspension was pipetted into individual glass tubes, frozen, and stored at −20 °C until needed. This source of thromboplastin was at least as effective as that obtained from human brain.

RVV-V was purified by the procedure described by Smith & Hanahan (1976).

*Methods. A. Factor V Assay.* This activity was assayed by the method of Kappeler (1955). In brief, this assay was conducted as follows. A 0.1-mL amount of baboon brain thromboplastin was mixed with 0.1 mL of factor V deficient plasma and 0.1 mL of diluted factor V sample immediately before starting the reaction. Clot formation was initiated by addition of 0.1 mL of 25 mM $CaCl_2$.

*B. Slab Gel Electrophoresis.* Gradient pore slab gel electrophoresis was done using PAA 2/16 and PAA 4/30 gels commercially available from Pharmacia. A recirculating buffer of 0.09 M Tris, 0.08 M boric acid, and 5 mM $CaCl_2$ was used at 8 °C and was preequilibrated with the gel for 15 min at 30 V/gel. The sample was applied in 5% sucrose and electrophoresed at 30 V/gel for 16 h. Purified factor V samples contained at least 2 units of factor V activity per track in a maximum volume of 50 $\mu$L. The gel was removed from the cassette immediately after electrophoresis and strained with 10% acetic acid–10% 2-propanol solution containing 0.1% Coomassie Brilliant Blue R for 3 h. Destaining was done by diffusion into the same solution without dye, usually for 1 day.

*C. Isolation Procedures for Factor V. 1. Bovine.* Bovine plasma factor V was isolated from bovine blood by the following procedure. Blood was collected by gravity, in the usual manner, from freshly killed animals into plastic containers. Usually a total of 2 L was obtained at one time. Within 30 s, the blood was distributed into 1-L plastic centrifuge bottles each containing 100 mL of 0.1 M sodium oxalate, with or without protease inhibitors. The bottles were filled to the 1-L mark, capped with the plastic lids, and mixed end over end. After transport to the laboratory at ambient temperatures, the erythrocytes were removed by centrifugation at 4 °C at 6000*g* in a Sorvall RC-3 centrifuge. Unless otherwise noted, all subsequent operations were conducted at 4 °C. The plasma was removed, mixed with $BaSO_4$ at a ratio of 100 g of $BaSO_4$ per L of plasma, gently stirred for 30 min, and centrifuged for 5 min at 6000*g*. The supernatant was collected and diluted 1:1 with deionized $H_2O$, the pH adjusted to 7.0 with 1 N HCl, and the factor V adsorbed onto 2-(hydroxypropyl)aminoethylcellulose (QAE-cellulose, 250 mL of swollen gel equilibrated overnight in 0.055 M calcium acetate, pH 7.25, per L of plasma). After the mixture was stirred for 20 min and allowed to stand for an additional 30 min, the cellulose was recovered by centrifugation at 6000*g* for 15 min. Then each 250 mL of QAE-cellulose was washed with 16–20 L of 0.055 M calcium acetate, pH 7.25, and the factor V eluted with 200 mL of 0.11 M calcium acetate, pH 7.25 (Smith & Hanahan, 1976).

This QAE eluate can be concentrated by use of poly(ethylene glycol) 4000 (PEG) precipitation or by Amicon TCF 10 ultrafiltration, using an XM 300 Diaflo membrane. The glycol precipitation was performed by adjusting the QAE eluate to pH 6.0 with acetic acid and adding 50% PEG dropwise with stirring to a final PEG concentration of 12% (w/v). After being stirred for 30 min, the mixture was centrifuged at 6000*g* for 10 min. The supernatant was removed, and the pellet was resuspended in 1–3 mL of buffer. This suspension can be dialyzed 2–4 h against additional buffer with an increased yield of factor V activity. PEG precipitation gave ~50% recovered activity after dialysis and ~25% recovery if the elution of the pellet was rapid. In contrast, ultrafiltration allows greater than an 85% yield of factor V activity. The factor V concentrate was then applied to a Bio-Gel A-5m column. Two different column sizes could be used: one measuring 1.6 × 55 cm and the other measuring 2.5 × 85 cm. The short column was used for more rapid recovery of factor V although some resolution was often sacrificed. Samples concentrated by ultrafiltration were applied in 0.11 M calcium acetate, pH 7.25, whereas samples concentrated by PEG precipitation were applied in Michaelis–25 mM $Ca^{2+}$ buffer, pH 7.35. In each instance, elution was accomplished by using Michaelis–25 mM $Ca^{2+}$ buffer. Fractions containing factor V activity were pooled and used directly for further study or concentrated by ultrafiltration and then examined.

*2. Human.* Human blood was collected into 0.1 volume of 0.1 M sodium oxalate in a 450-mL blood bag thoroughly rinsed free of all citrate anticoagulant. This was centrifuged at 4 °C at 6000*g* for 10 min in a Sorvall RC-3 centrifuge, and the procedure described for bovine blood was followed with a few modifications to improve the yield of factor V. These are briefly summarized below.

Subsequent to removal of $BaSO_4$, the plasma was diluted as before but no pH adjustment was made. Also, when PEG concentration was used, no pH adjustment was made. With no adjustment in pH, recovery of activities was equal to or better than that obtained with bovine factor V. At pH values lower than 7.0, almost complete loss of human factor V was noted. This was not the case with the bovine source, which proved stable under these conditions.

## Results

*General Comments on Factor V Preparations.* A comparison of two bovine Factor V samples, one collected in DFP and the other collected with the usual protease inhibitors, is shown in parts a and b of Figure 1. These column chromatographs clearly show that the presence of 2 mM DFP in the anticoagulant at collection time had a significant effect on the apparent molecular weight of the isolated factor V. This higher molecular weight factor V cannot be preserved by addition of other commonly used inhibitors such as STI (25 $\mu$M), PMSF (10 mM), $\epsilon$-aminocaproic acid (100 mM), or heparin (2890 USP units/L of whole blood), nor can addition of 2 mM DFP several minutes after collection give assurance of isolation of a higher molecular weight factor V. These

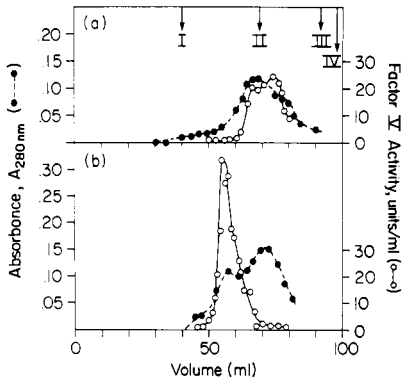


FIGURE 1: Chromatographic behavior of bovine plasma factor V on Bio-Gel A-5m. (a) Factor V isolated from bovine blood collected in 0.1 M sodium oxalate containing 10 mM PMSF but no DEP. The 1.6 × 52.5 cm column was eluted in 1.2-mL fractions at a flow rate of 20 mL/h. A similar profile was obtained when benzamidine, STI, and/or ε-aminocaproic acid was used in the absence of DFP. (b) Factor V isolated from bovine blood collected in 0.1 M sodium oxalate plus DFP at a final concentration of 2 mM. Roman numerals refer to the elution volumes for molecular weight markers: I, Blue Dextran (2 000 000); II, apoferritin (450 000); III, catalase (240 000); IV, aldolase (158 000). Column conditions were identical with those of (a).

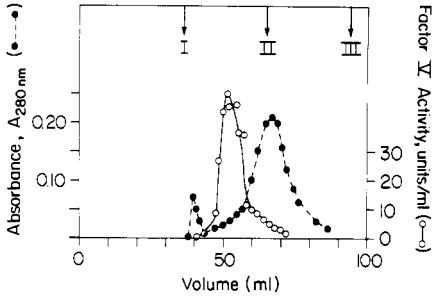


FIGURE 2: Chromatographic behavior of human plasma factor V on Bio-Gel A-5m. Factor V was isolated from human blood collected in 0.1 M sodium oxalate but with no protease inhibitors present. Column size and elution conditions were the same as those described in Figure 1 and the text.

results and the observation that factor V collected with these inhibitors can still be activated to a certain extent by thrombin, for example, suggest that this molecule is sensitive to limited proteolysis at the initial step in purification, probably before the erythrocytes and other formed cells can be removed from the anticoagulated blood. Further, it has been observed that high molecular weight factor V maintains its apparent size when rechromatographed in low salt (0.025 M NaCl) or in high salt (1.0 M NaCl) media. At least with bovine factor V, the apparent size of the high molecular weight factor V remains the same even with changes in pH, from pH 6.0 in the PEG precipitation step to pH 7.5 in 0.2 M Tris–10% glycerol buffer. Also, high molecular weight factor V has been isolated from the plasma of human blood collected by venipuncture. Human factor V, when chromatographed on Bio-Gel A-5m (Figure 2), yielded a molecular weight of $1 \times 10^6$, not unlike that of the high molecular weight bovine factor V.

Table I illustrates the purification procedure employed for isolation of bovine factor V. It is evident that this approach yields moderate amounts of highly purified, large molecular weight material. By use of a similar approach, high molecular weight factor V can be obtained from human plasma, which contained an average starting value of 1–2 units/mL, in ~25% yield. However, the (purified) human factor V had a much lower specific activity, i.e., 37.5 units/mg of protein, than found for the bovine source, i.e., 116 units/mg of protein. Particularly important to the success of this isolation scheme was the concentration step, since factor V must be removed from rather large volumes prior to the final concentration step. To date, it has been possible to obtain a good yield of human factor V after PEG precipitation and resuspension if no pH adjustment is made during the process; i.e., the pH must not drop below 7.0. Only a slight change in yield is observed in the bovine samples over the pH range from 6.5 to 8.0. Of more importance, both human and bovine factor V can be recovered in yields of 75% or greater, using the XM 300 membrane ultrafiltration technique. In general, this has been the preferred route to volume reduction of the bovine preparation.

Table I: Purification Profile of Factor V from Bovine Plasma Collected in the Presence of 2 mM DFP

| step | vol (mL) | total units of act. | protein[a] (mg/mL) | sp act. (units/mg of protein) | yield (%) |
|---|---|---|---|---|---|
| plasma | 452 | 1175 | 20.54 | 0.12 | 100 |
| $BaSO_4$ eluate | 430 | 993 | 14.10 | 0.16 | 84 |
| QAE eluate | 118 | 422 | 1.23 | 2.90 | 36 |
| XM 300 concentrate[b] | 14 | 434 | 11.3 | 2.74 | 37 |
| Bio-Gel A-5m pool | 61 | 275 | 0.039 | 115.6 | 23 |

[a] Determined from the $A_{280}$ value. [b] This concentration approach provided an excellent route to handling large volumes of factor V containing solutions. Even though foaming might occur, recoveries of total activity units were greater than 50%. Only 4 mL of this concentrate was applied to a 2.5 × 85 cm Bio-Gel A-5m column. The yield from column chromatography is calculated by assuming that all 14 mL of the XM 300 concentrate was applied.

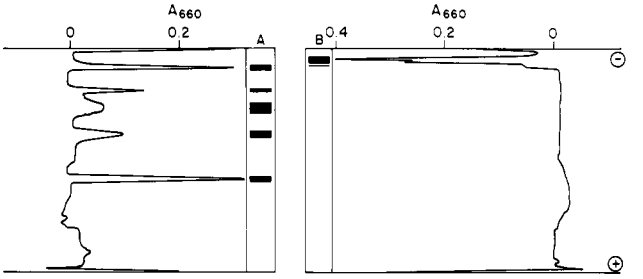


FIGURE 3: Electrophoresis pattern of purified bovine factor V on gradient pore slab gels. A densitometric tracing of globular protein standards (A) is compared with a tracing of 8 units of bovine factor V (B) from the activity peak of a Bio-Gel A-5m column. Both samples were removed from a single slab gel (in this case a PAA 4/30 gel) for scanning of Coomassie-stained material at 660 nm. A facsimile of each gel is given to illustrate the high molecular weight nature of purified factor V. Protein standards and their molecular weights in order from top (−) to bottom (+) are the following: bovine thyroglobulin, 669 000; ferritin, 440 000; catalase, 242 000; LDH, 140 000; BSA, 67 000. Conditions for electrophoresis are given in the text.

Figure 3 illustrates two samples applied to a Pharmacia PAA 4/30 gradient pore slab gel. Sample A contains five globular protein standards (see figure legend). Sample B contains a fraction of purified bovine factor V collected with 20 mM DFP in the anticoagulant. This fraction represents the peak of factor V activity eluting from a Bio-Gel A-5m column. Densitometric scanning of stained material at 660 nm reveals a closely spaced doublet in the factor V sample. Relative to protein standards, the approximate size of this doublet is 820 000 and 760 000 daltons, respectively. To date, it has not been possible to determine whether one or both species contributed to measurable factor V activity or whether one molecule is a contaminant or degradation product of the other.

While every effort was made to complete the entire isolation and purification procedure within 36 h of blood collection, the

stability of the human and bovine factor V samples on storage has not proven outstanding. Nonetheless, bovine factor V obtained from the Bio-Gel A-5m columns could be maintained without a significant decrease in size for up to 7 days when stored at 4 °C in Michaelis–$Ca^{2+}$ buffer. However, under similar conditions both purified bovine and human factor V start to decay in activity after some 2 to 3 days. At this point in time, no explanation for these changes can be offered.

A total of 20 different collections of bovine plasma have been processed in the presence of 2 mM DFP, and in 95% of the samples the purified factor V behaved as a molecule with an average molecular weight of 1 000 000 with a range of values from 800 000 to 1 200 000. In a comparable number of runs with human blood (usually of the order 250–500 mL at a time), a similar range of values was noted. In the case of the bovine samples, if a particularly clean incision was made in the jugular vein of the animals, one could isolate in the absence of DFP a similar high molecular weight (~1 000 000) factor V.

*Activation of High Molecular Weight Factor V.* Incubation of bovine or human high molecular weight factor V with thrombin (~1 μg/4 units of factor V) or purified activator from RVV (~1.5 μg/3 units of factor V) at pH 7.35 at 25 °C resulted in significant activation of factor V. In the case of bovine plasma factor V, the extent of activation was some 10–15-fold, whereas with the human factor V the degree of activation was on high as 50-fold under these conditions. Though only limited attention was paid to the nature of the factor Va derived by these agents, it was evident from chromatographic examination by Bio-Gel A-5m and Sepharose CL6B that the activated forms were of a lower molecular weight, with minimum values of 400 000–500 000 being obtained. These studies are continuing and will be reported elsewhere.

## Discussion

The evidence presented here clearly shows that a high molecular weight factor V, in the range of 800 000–1 000 000, can be isolated from (slaughterhouse) bovine blood if a high level, 2 mM, of the serine protease inhibitor, DFP, is included in the anticoagulant mixture at the time of collection. Unless this precaution is followed at this point in the isolation procedure (bovine) factor V of molecular weight less than 500 000 is obtained even in the presence of the usual protease inhibitors, e.g., soybean trypsin inhibitor, *p*-(chloromercuri)benzoic acid, and phenylmethanesulfonyl fluoride plus low concentrations (~0.1 mM) of DFP. Interestingly, collection of cow blood by venipuncture yielded the high molecular weight (~1 000 000) factor V even in the absence of DFP or other inhibitors. This would suggest that a tissue protease is involved in causing conversion of high molecular weight factor V (~1 000 000) to the commonly observed lower molecular weight species. It will be of obvious import to isolate and identify this enzyme.

Our results are somewhat comparable to those reported by Colman and his colleagues (Saraswathi et al., 1978), who noted that a high molecular weight factor V could be isolated from bovine blood collected by venipuncture. They proposed that proteolytic degradation might give rise to the lower molecular weight forms seen by several investigators but did not elaborate further on this point. Recently, Esmon (1979) commented on the presence of a high molecular weight species, i.e., 850 000, by Bio-Gel A-1.5m chromatography in this bovine factor V preparation but also did not pursue this observation. Of considerable interest and import, our study has shown also for the first time that factor V of human plasma can be isolated with an apparent molecular weight of 1 000 000.

In addition to the observation of the effectiveness of DFP in this isolation, the availability of Bio-Gel A-5m for column chromatographic purifications made isolation of the $1 \times 10^6$ species from human as well as bovine plasma a possibility. While Sepharose CL6B was effective also, it was not as consistent in its behavior as was the Bio-Gel A-5m.

An immediate concern arose upon finding of this very high molecular weight factor V and this centered on the possibility that this represented an aggregated form of the lower molecular weight species. Several arguments could be mounted to counter this possibility. An important supporting observation was that DFP preserved the high molecular weight form especially during the initial collection phase. However, it should be noted that even in the presence of DFP, low molecular weight factor V can be found if there is a sufficiently large amount of tissue involvement in the blood collection process. Further, it has been observed that high molecular weight factor V maintains its apparent size when rechromatographed in low salt (0.025 M NaCl) or in high salt (1.0 M NaCl) media. At least with bovine factor V, the apparent size of the high molecular weight factor V remains the same even with changes in pH, from pH 6.0 in the PEG precipitation step to pH 7.5 in 0.2 M Tris–10% glycerol buffer. The fact that high molecular weight factor V is obtained by chromatography of undiluted plasma collected in oxalate alone (from blood collected by venipuncture) would eliminate it as an artifactual form. Finally, recent evidence in this laboratory (J. Biswas, unpublished experiments) has shown that an antibody raised against the high molecular weight (1 000 000) bovine factor V does not cross-react with the lower molecular weight (400 000) species.

One cannot help but be impressed even by causual reading of the literature on factor V of the variations in molecular weights reported by laboratories well versed in protein isolation. It appears likely that in most cases, the presence of a protease, highly sensitive only to DFP, was responsible for formation of a low molecular weight, i.e., 500 000 and less, factor V activity. It would seem probable that this protease is derived from tissue injury, since collection of blood by venipunture allows recovery of the high molecular weight ($1 \times 10^6$) form. However, other conditions can have impact on the character of the final factor V product as exemplified in a recent publication by Bolhuis et al. (1979). In this study, human plasma factor V with a molecular weight of 296 000 was obtained by an isolation procedure in which blood was collected in citrate and a freeze–thaw step was employed. It has been our experience that similar treatments of factor V samples led to complete inactivation, very low recoveries, and/or conversion to an activated factor V.

Our findings of a high molecular weight factor V will require reinvestigation into various facets of its biochemical behavior, particularly its conversion to an "activated" form and its interaction with phospholipids in in vitro systems. Though it was not our intent here to prove the unique purity of our high molecular weight factor V, current studies with antibody preparations appear likely to produce high-purity material. We have also made a preliminary determination that this preparation is free of detectable factor VIII related antigen and factor VIII coagulant activity. The observations reported here will help in allowing a uniform type of factor V to be available to investigators in this field.

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# Nuclear Envelope of Chinese Hamster Ovary Cells. Re-formation of the Nuclear Envelope following Mitosis†

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ABSTRACT: We have developed a technique for isolating nuclei and nuclear envelope(s) (NE) from Chinese hamster ovary (CHO) cells which does not depend on the use of detergents to solubilize contaminating cytoplasm. In our procedure NE are prepared from purified nuclei by nuclease digestion and subsequent high salt–sucrose gradient centrifugation. The nuclei and NE fractions are free of significant contamination by other subcellular organelles as judged by electron microscopy and enzyme analysis. Examination of the peptide and glycopeptide composition of the NE fraction by sodium dodecyl sulfate–polyacrylamide gel electrophoresis reveals a very complex coomassie blue staining profile with prominent bands in the 55 000–75 000 molecular weight range. Using this NE isolation technique, we have examined the breakdown and re-formation of the NE during a limited stage (late $G_2$, M, and early $G_1$) of the replicative cycle in synchronized populations of CHO cells. Our data demonstrate that a minimum of 60% of the early $G_1$ NE protein and a minimum of 50% of the early $G_1$ NE phospholipid were present in the cell during the preceding $G_2$ phase of the cell cycle and were reutilized in the re-formation of the NE occurring during late M and early $G_1$. Our evidence suggests that the vast majority of the newly synthesized peptides and glycopeptides of the NE which appear in the daughter NE are synthesized during the early $G_1$ phase of the replicative cycle. Examination of the NE peptides by one-dimensional gel electrophoresis suggests that no reproducible changes in NE peptide composition can be correlated with specific phases of the cell cycle.

The NE has been shown by a variety of microscopic techniques to break down and re-form during cell division in most species of plants and animals [for a review, see Franke & Scheer (1974)]. Unfortunately, very little is known about the fate of the NE during mitotic breakdown nor is there good experimental data concerning the origin of the components which reform the NE in late M and early $G_1$. Similarly, the mechanism(s) whereby the NE is disassembled at the initiation of M and re-formed in late M has not been elucidated.

It has been suggested by a number of workers (Porter & Macado, 1960; Moses, 1964; Robbins & Gonatas, 1964; Murray et al., 1965; Brinkley et al., 1967) that, following NE breakdown, fragments of NE mingle with and become indistinguishable from the endoplasmic reticulum (ER). Furthermore, it has been argued that components of the ER are utilized in the re-formation of the NE which occurs during late M–early $G_1$ (Porter & Macado, 1960; Moses, 1964; Flickinger, 1974). Other investigators have suggested that the NE or components of the NE persist through mitosis as distinct cytoplasmic entities which are biochemically different from the ER. It has been suggested that these hypothetical cytoplasmic components are specifically reutilized to re-form the NE at the completion of M (Erlandson & DeHarven, 1971; Scott et al., 1971; Maruta & Goldstein, 1975; Maul, 1977). Finally, some workers in the field have suggested that the NE which reappears at the end of M is a product of de novo synthesis of all the NE components (Jones, 1960).

To study the composition and biosynthesis of the intact NE and to examine biochemical changes which might occur in the NE during the cell cycle, it was necessary to develop a technique which would permit the isolation, from cultured cells, of NE containing the inner and outer membranes of the NE as well as the pore–lamina complex (Aaronson & Blobel, 1974; Riley et al., 1975; Dwyer & Blobel, 1976; Hodge et al., 1977).

Using a NE isolation technique dependent on nuclease digestion of purified nuclei followed by high salt–sucrose gradient centrifugation, we have investigated the breakdown and re-formation of the NE during the $G_2$–M–$G_1$ transition in CHO cells. Our data strongly suggest that the majority of NE peptides and phospholipids which are found in the early $G_1$ NE were present as cell components prior to the initiation

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